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ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

Twenty-third Quarterly Report of Progress

Research Project R-36-015-001

October 1, 1970 - December 31, 1970

Conducted by

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ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

Introduction

In this quarter, we continued our investigation in identifying the thermal inactivation curve of Bacillus subtilis var. niger spores with 500 μg of water per ml of headspace. We also tested the hardware and equipment to be used for evaluating a terminal process for unmanned landers. This equipment was subsequently delivered and installed at Cape Kennedy.

I. THERMAL INACTIVATION STUDIES

Several experiments were conducted to determine the nature of the thermal inactivation curve of B. subtilis var. niger spores under 0.25, 2.6, 10, and 100 μg of water per ml of headspace. The conventional plate count method was used in assaying spore survivors ranging from 10^6 to $> 10^0$ spores per cup. Figures 1, 2, and 3 show a composite of each determination. Data from these determinations were similar and the results were reproducible. Figure 4 shows the results with 100 μg of water per ml of headspace. The results showed a significant variation between two runs. Likewise, we investigated the spore survival curve with 500 μg of water per ml of headspace. Our experimental results by the plate count method showed significant variations within shelf samples.

In our standard procedure, the spores were suspended in 95% ethyl alcohol and diluted in sterile double distilled water and were dispensed with a microburette in 0.01 ml amounts in stainless

steel cups to give about 10^6 spores per cup. The cups were arranged on circular shelves and placed in 206 x 300 tin cans. Thirty cups were on each shelf and four shelves were used in each can for a total of 120 cups per can. The cans, lids, and contents were dried in a vacuum oven for 90 minutes at 45 to 50°C (at 1.5-inch Hg pressure absolute). To increase the drying rate, the oven was purged with dry nitrogen every 10 minutes for the first 70 minutes, and this was followed by five consecutive purges of nitrogen with a vacuum cycle between each purge. After drying, the cans, lids, and contents were removed from the oven and cooled to about 30°C in the equilibration hood. A weighed amount of sodium tungstate dihydrate was placed in the bottom of each can and the cans were removed from the equilibration hood, and at designated time intervals they were dropped in a 75°C oil bath and preconditioned for 5 hours. After preconditioning the cans were heated immediately in a 125°C oil bath at various time intervals and cooled in a refrigerated bath.

Spore survivors from cups containing about one spore per cup or greater were assayed by sonifying the cups in peptone water and plating.

II. STUDIES ON A TERMINAL STERILIZATION CYCLE

The hardware and equipment to be used for the evaluation of a terminal sterilization process for unmanned landers were assembled.

In the experimental setup, the protocol is as follows: (a) stainless steel cups will be contaminated with bacterial spores; (b) the

cup will be exposed to a typical terminal sterilization process; (c) broth will be added to each cup and will be incubated for 7 days at 32°C; (d) the cup will be scored for either the presence or absence of microorganisms; and (e) the resulting data will be evaluated in terms of an upper limit of effectiveness based on the number (4,000-10,000) of cups assayed.

Preliminary testing of the hardware has been completed. We have demonstrated that it is possible to place cups in the oven, go through a heat cycle, and remove the cups from the oven without measurable contamination. This test was conducted with 720 cups, and we found only one positive cup.

We have also conducted two experiments regarding questions of sample independence of test cups.

About 720 cups were used in each experiment. Thirty cups were arranged in circular stainless steel trays. All these cups were inoculated with 0.01 ml spore inoculum per cup, dried in a vacuum oven, and then placed in the 125°C oven. The oven has three shelves, and each shelf holds 24 30-cup trays. These trays were arranged in four rows with six trays per row.

Previous data for B. subtilis var. niger were used to predict the holding time at 125°C that would yield around one organism per cup. The trays were heated, cooled, and then the broth placed in each cup. The cups were scored for growth or no growth. An MPN/cup was estimated, and these values are given in Table 1 for the four rows on the three shelves.

Tests for equality of row means showed that a significant difference occurred at the $\alpha = 0.01$ level. The two middle rows were consistently lower than the two outer rows. In both sets the maximum value was about three times the minimum. These preliminary tests indicated that some adjustments would be necessary once the oven was installed at Cape Kennedy.

TABLE 1

Summary of MPN Estimates in 12 Positions

Position	MPN/cup			
	1	2	3	4
Set I				
Top	3.0	0.9	0.8	1.6
Middle	1.7	0.8	0.9	1.9
Bottom	2.2	1.2	0.9	1.6
Set II				
Top	4.5	2.1	2.3	4.1
Middle	2.4	1.6	2.0	2.9
Bottom	2.8	1.6	2.4	3.0

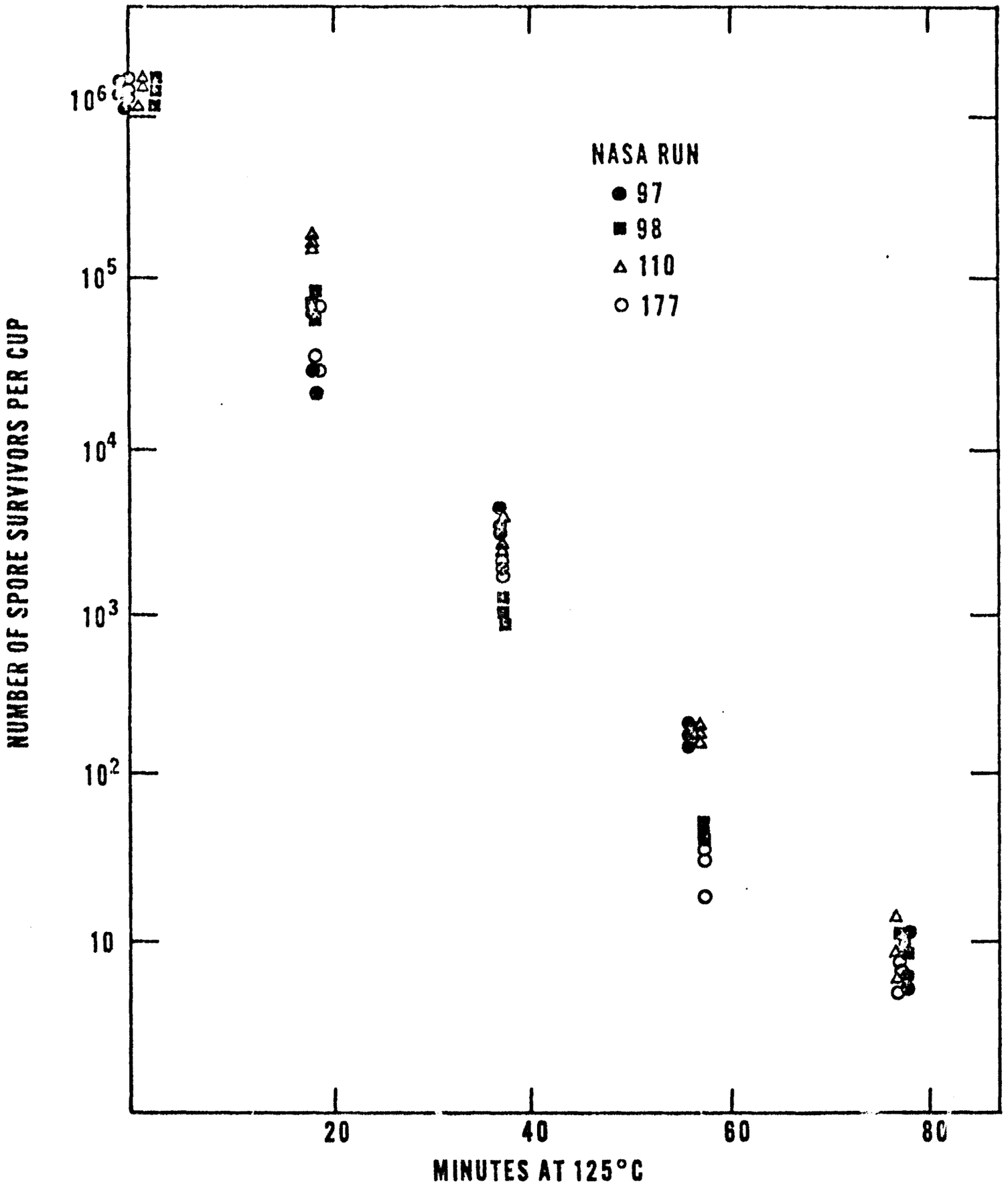

Figure 1. Thermal inactivation of B. subtilis var. niger spores at 125°C and 0.25 µg water per ml.

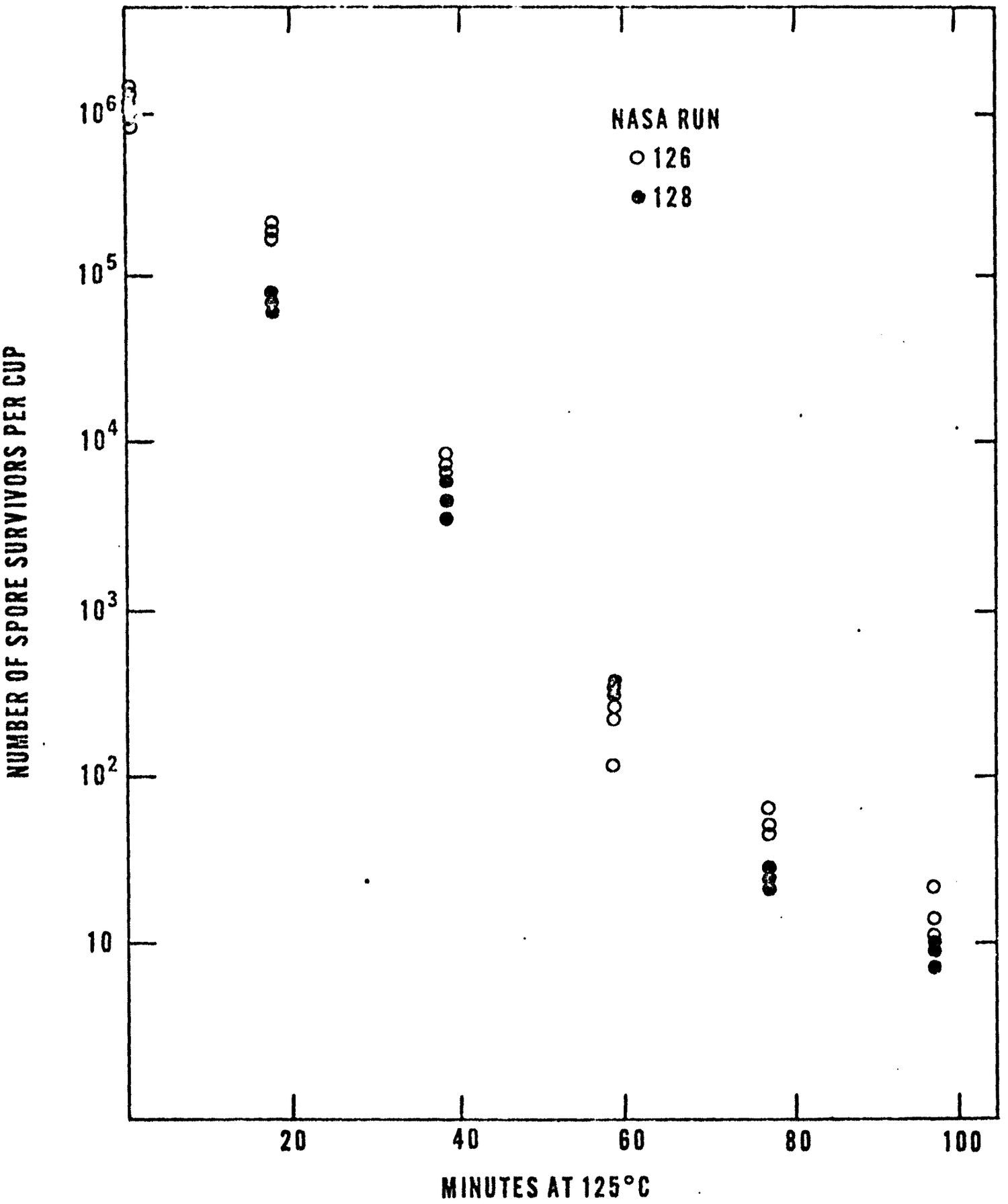

Figure 2. Thermal inactivation of B. subtilis var. niger spores at 125°C and 2.6 µg water per ml.

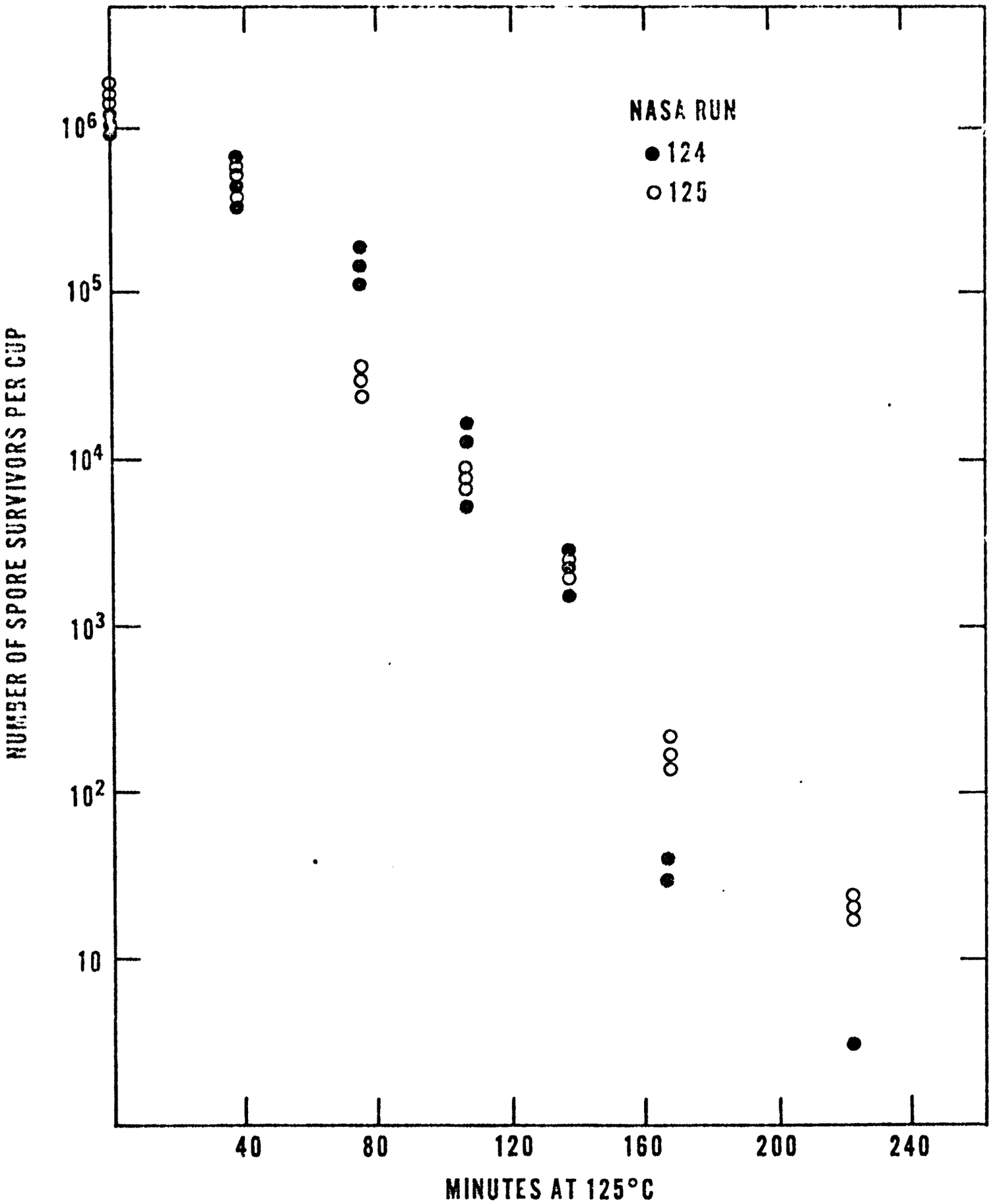

Figure 3. Thermal inactivation of B. subtilis var. niger spores at 125°C and 10.0 µg water per ml.

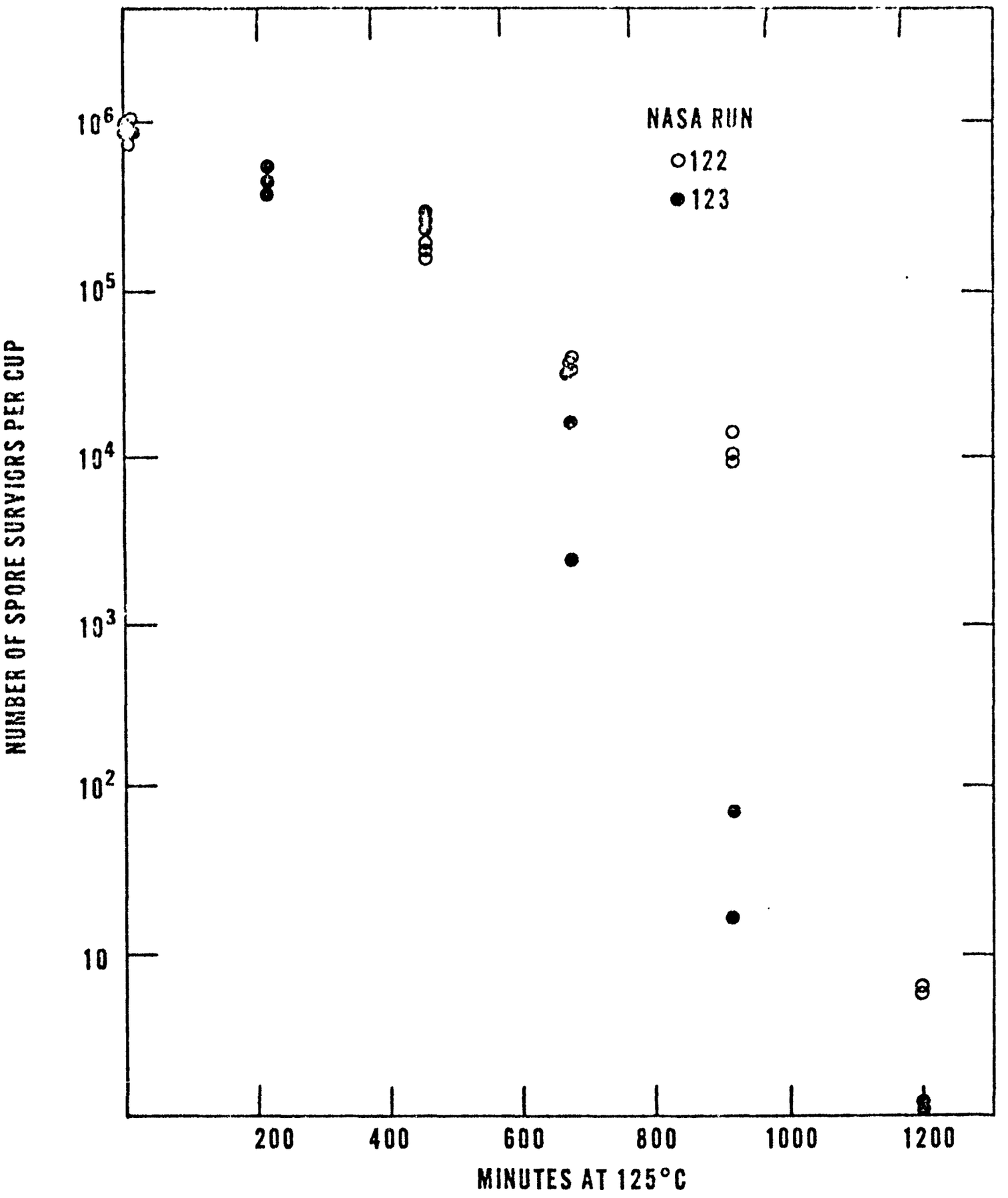

Figure 4. Thermal inactivation of B. subtilis var. niger spores at 125°C and 100.0 µg water per ml.